A BACTERIAL LEAF SPOT OF ZINNIA IN NEW SOUTH WALES

A. L. Bertus
Biological and Chemical Research Institute, Department of Agriculture, Rydalmere, New South Wales, 2116

AND

A. C. Hayward
Department of Microbiology, Medical School, University of Queensland, Herston, Brisbane, 4006

(Plates vii, viii)

[Accepted for publication 24th March 1971]

Synopsis

A bacterial disease of zinnia caused by *Xanthomonas nigromaculans* f. sp. *zinniae* is recorded for the first time in Australia. Two isolates from material collected at Armidale, N.S.W., were pathogenic on inoculation into the zinnia cultivar from which they were originally obtained and to eight other cultivars. These cultures were compared with two isolates of the species from Africa, including the original culture of Hopkins and Dowson (1949), and were shown to be indistinguishable except for some minor differences in carbohydrate utilization.

Introduction

In February 1969, leaves of zinnias growing in the grounds of Duval College, University of New England, Armidale, were observed to have irregular blackened spots from 1 mm. in diameter to large necrotic areas over 20 mm. across. The larger areas seemed to be formed by the coalescing of several small spots. The spots had a water-soaked appearance and all lesions were surrounded by yellow chlorotic margins (Plate vii, Fig. 1). A massive bacterial ooze was obtained from some of the spots on suspension in water. The plants were grown from commercial seed of the cultivar Giant Dahlia Flowered Mix.

The only earlier record of a bacterial leaf disease of zinnia in New South Wales is from Cremorne in December, 1948 (Anon., 1949). This specimen is filed in the Biology Branch Herbarium at Rydalmere (DAR 4007). The leaves show small black spots ; it is noted on the herbarium packet that abundant ooze was observed on the spots. The organism was not identified.

A survey of available plant disease check lists of other Australian States does not reveal any published record of a bacterial leaf disease of zinnia.

A bacterial leaf spot and flower disease of zinnia was reported by Hopkins and Dowson (1949) from Southern Rhodesia. The causal bacterium was described by them and named *Xanthomonas nigromaculans* f. sp. *zinniae*. The host symptoms described and illustrated closely resemble the symptoms observed at Armidale. This disease has also been recorded from Brazil (Robbs, 1954) and from Sierra Leone (Deighton, 1956).

In the following an account is given of the isolation and identification of the causative bacterium, which involved pathogenecity tests, cultural and physiological observations and electron microscopy. This detailed study was undertaken as the descriptions by Hopkins and Dowson (1949) and others are inadequate.

Materials and Methods

Isolation and cultural and physiological tests: Water-soaked spots were cut from leaves and suspended in 3 ml. of sterile distilled water; after standing for two hours at room temperature to allow egress of bacterial ooze, loopfuls of suspension were streaked out on 2% sucrose/peptone agar (Hayward, 1964).

Two isolates of *Xanthomonas nigromaculans* f. sp. *zinniae*, NCPPB 189 isolated from *Zinnia* sp. in Southern Rhodesia in 1946 and NCPPB 799 isolated from *Zinnia elegans* in Malawi in 1960, were obtained under licence from the National Collection of Plant Pathogenic Bacteria, Plant Pathology Laboratory, Hatching Green, Harpenden, Herts., England. This species is the only known bacterial pathogen affecting the leaves of *Zinnia*. The two cultures were compared with two isolates obtained from the Armidale collection, accessioned as 0365a and 0365b.

Cultural and physiological tests for identification were carried out as described by Allen, Hayward, Halliday and Fulcher (1970). All test media were incubated at 28° C. unless otherwise indicated. Yellow pigments from isolates of the bacterium were extracted and saponified using the techniques described by Starr and Stephens (1964). Solutions of the yellow pigments in petroleum ether were examined in a Perkin-Elmer 4000A, UV–VIS recording spectrophotometer.

Pathogenicity tests: Two isolates of the bacterium were grown on potato dextrose agar and a suspension in sterile distilled water was obtained by washing off the growth of six one-week-old slopes of each isolate in 50 ml. of water. The suspensions were atomized on 18-day-old seedlings from the same lot of seed from which the plants showing natural infection were grown. Controls were sprayed with sterile distilled water. Each group of plants was isolated in separated humid chambers. Plants of eight other cultivars grown from commercial seed were inoculated in the same way with one isolate of the bacterium.

Observations

Isolation and cultural characteristics: After 48 hours incubation at 28° C., the cultures originating from suspensions of ooze from leaf spots showed a glistening yellow growth accompanying a mixture of faster-growing bacterial colonies which were visible to the naked eye after 24 hours incubation. The slower growing yellow colonies were uniform in appearance. Colonies were picked off and purified by successive re-streaking. After three days incubation colonies of the yellow, slow-growing bacterium were 3·0–3·5 mm. in diameter, convex, circular with an entire edge, amorphous, glistening, viscid and readily emulsified in water. On a peptone/yeast extract medium lacking carbohydrate the colonies were 1·5–2·0 mm. in diameter after four days incubation at 28° C.; the colonies were low convex and markedly less mucoid than on the carbohydrate-containing medium. In cultural characteristics, therefore, the bacterium resembled the xanthomonads.

Cultural and physiological tests: The four cultures 0365a, 0365b, NCPPB 189 and NCPPB 799 were alike in being aerobic, Gram-negative, uniformly stained rods, actively motile with a single polar flagellum (Plate VIII), which produced yellow viscid growth on 2% sucrose/peptone agar. All cultures were oxidative in their metabolism of carbohydrates and produced acid under aerobic conditions from cellobiose, fructose, galactose, glucose, glycerol, lactose, maltose, sucrose and trehalose. NCPPB 189 produced acid from mannitol; the other three cultures were negative after 14 days incubation. Arabinose was oxidized promptly by NCPPB 189 and slowly by NCPPB 799 and 0365b; 0365a failed to oxidize arabinose. No acid was produced after 14 days incubation from the

following carbohydrates : adonitol, dulcitol, inositol, salicin and sorbitol. The following tests were positive for all cultures : aesculin hydrolysis, phosphatase, malonate utilization in Leifson's medium, citrate utilization in Simmons' medium, growth in a peptone/yeast extract medium containing 2% sodium chloride, and in the same medium containing 3% sodium chloride. All cultures were strongly lipolytic on Tween 80 agar and gave wide zones of hydrolysis on starch agar and on gelatin agar. Twenty-four-hour peptone/yeast extract agar cultures were tested for catalase and for oxidase using Kovacs' method ; all cultures were catalase-positive and gave a weak reaction in the Kovacs test, in which there was a colour change at 5–15 sec. after application of culture to the reagent-impregnated filter paper. Pectate gel medium was progressively softened at 7–28 days. Raw potato slices 10 mm. thick, heavily inoculated with each of the four cultures down a narrow median groove and incubated for 24 hours at 28° C. showed a golden to brownish band of growth and rot extending about 5 mm. on either side of the groove. The rot did not extend significantly after a further 24-hour incubation. This confirms the pectolytic activity of the species and perhaps indicates that this pathogen is potentially capable of producing a soft wet rot in plant tissues. The capacity to rot potato slices is fairly common in *Xanthomonas*. The following tests were uniformly negative : production of nitrite from nitrate, production of gas from nitrate, sulphatase, oxidation of gluconate, growth in peptone/yeast extract medium containing 6% sodium chloride, and in the same medium containing 10% sodium chloride, production of phenylpyruvic acid from phenylalanine, arginine dihydrolase, chitin hydrolysis, levan production on 5% sucrose/peptone agar, production of 3-keto-lactoside from lactose. No diffusible pigments were produced on any medium. The extracted yellow pigments of cultures 0365a and 0365b were found to have the same absorption maxima claimed by Starr and Stephens (1964) to be characteristic of a carotenoid alcohol found only in xanthomonads.

Pathogenicity tests : All inoculated plants of Giant Dahlia Flowered Mix showed infection after 14 days. The controls sprayed with water remained healthy. The symptoms were similar to those found in natural infection (Plate VII, Fig. 2) and similar to published photographs (Hopkins and Dowson, 1949). There was no obvious difference between the reactions of the two isolates. The bacterium was readily re-isolated from infected plants.

All plants of the other eight cultivars of zinnia inoculated with one isolate of the bacterium became infected and the bacterium was re-isolated in each instance. The cultivars were Thumbelina Mixed, Persian Carpet, State Fair Tetraploid Mixed, Sprite Mixed, Semi-dwarf Coquette Mixed, Lilliput Mixed, Pink Buttons and Linearis.

Discussion and Conclusions

On symptoms the zinnia disease observed at Armidale appeared similar to that originally described by Hopkins and Dowson (1949) from Southern Rhodesia and subsequently found in Brazil (Robbs, 1954) and Sierra Leone (Deighton, 1956).

The cultural and physiological properties of the two isolates from zinnia leaves from Armidale are wholly consistent with the genus *Xanthomonas* as described by Dye (1962) and others. These cultures closely resemble the two cultures from Africa and differ only in the fact that alone of the four cultures NCPPB 189 oxidized both mannitol and arabinose promptly. The differences in carbohydrate utilization are trivial and within the range of variation which is encountered in other species of the genus *Xanthomonas*. There is no doubt that the two Armidale cultures are correctly identified as *Xanthomonas nigromaculans* (Takimoto) Dowson 1943 f. sp. *zinniae* Hopkins and Dowson 1949.

ACKNOWLEDGEMENTS

The support of a research grant to one of us (A.C.H.) from the Rural Credits Division of the Reserve Bank of Australia is gratefully acknowledged. We thank Mr. J. V. Hardy and Mr. J. A. Fuerst for their help in the preparation of the electron micrographs which were taken at the Electron Microscope Centre, University of Queensland, St. Lucia; Dr. A. B. Lloyd, University of New England, Armidale, for specimens of diseased zinnias and zinnia seed; and Mr. A. Searle, Department of Agriculture, Rydalmere, for the photographs on Plate VII.

References

ALLEN, R. N., HAYWARD, A. C., HALLIDAY, W. J., and FULCHER, J., 1970.—Bacterial blight of *Vicia sativa*: aetiology of the disease and identification of the pathogen. *Aust. J. biol. Sci.*, 23: 597–606.

ANON., 1949.—New plant diseases. *Agric. Gaz. N.S.W.*, 60: 141–142.

DEIGHTON, F. C., 1956.—Plant Pathology Section. *Rep. Dep. Agric. S. Leone 1954*, 27–28. (Abstr. in *Rev. appl. Mycol.*, 36: 381, 1957.)

DYE, D. W., 1962.—The inadequacy of the usual determinative tests for the identification of *Xanthomonas* spp. *N.Z. J. Sci.*, 5: 393–416.

FUERST, J. A., and HAYWARD, A. C., 1969.—Surface appendages similar to fimbriae (pili) on *Pseudomonas* species. *J. gen. Microbiol.*, 58: 227–238.

HAYWARD, A. C., 1964.—Characteristics of *Pseudomonas solanacearum*. *J. appl. Bact.*, 27: 265–277.

HOPKINS, J. C., and DOWSON, W. J., 1949.—A bacterial leaf and flower disease of Zinnia in Southern Rhodesia. *Trans. Br. mycol. Soc.*, 32: 252–254.

ROBBS, C. F., 1954.—Bacterias fitopatogenicas do Brasil (Phytopathogenic bacteria of Brazil). *Agronomia, Rio de J.*, 13: 265–282. (Abstr. in *Rev. appl. Mycol.*, 35: 752, 1956.)

STARR, M. P., and STEPHENS, W. L., 1964.—Pigmentation and taxonomy of the genus *Xanthomonas*. *J. Bact.*, 87: 293–302.

EXPLANATION OF PLATES

PLATE VII

Fig. 1. Bacterial leaf spot of zinnia. Natural infection, Armidale, N.S.W. ×0·6.

Fig. 2. Bacterial leaf spot of zinnia. Artificial inoculation with isolate 0365b. ×1·2.

PLATE VIII

Fig. 1. *Xanthomonas nigromaculans* f. sp. *zinniae* NCPPB 189. ×24,540. Preparation negatively stained with 0·5% ammonium molybdate. A single polar flagellum is shown.

Fig. 2. *Xanthomonas nigromaculans* f. sp. *zinniae* 0365. ×8,180. Preparation negatively stained with 0·5% ammonium molybdate. Single polar flagella are shown.
[The technique of negative staining used was that described by Fuerst and Hayward (1969).]